DEVELOPMENT OF HYBRID ANTIBACTERIAL MEMBRANE BY INCORPORATING SILVER PARTICLE WITH 2,4,6-TRIAMINOPYRIMIDINE AS COMPATIBILIZER

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Chemical Engineering)

Faculty of Chemical Engineering
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ACKNOWLEDGEMENTS

I wish to thank Allah Almighty who granted me the good health to pursue this research. I give Him my heartfelt gratitude for the strength especially when I was weak. The divine wisdom and favours received from Allah are too numerous to count.

I am grateful to my supervisor Prof. Dr. Ahmad Fauzi Ismail for his continuous encouragement, brilliant ideas, motivation and moral support in supervising me throughout the program. I am also grateful to Prof. Madzlan Aziz, Emiratus Prof. T. Matsuura and Prof K. Nagai for their encouragement and good wishes for my work.

I am also grateful to the technical advisors Mr Mohd Sohaimi Abdullah and Mr Ng Be Cheer for their effort and time.

I want to thank all my colleagues in AMTEC from the smallest to the greatest for supporting this PhD work: Dr. Lau Woei Jye, Dr Muhammad Noorul Annam, Dr. Suhaila Mohd Sanip, Erna Yuliwati, Juhana Jaafar, Noor Haniza Yusof, Goh Pei Sean, Agung Mataram, Nur Atiqah Abd Hamid, Dayang Salyani Abang Mahmod, Muhammad Abdul Razis Saidin for their help, encouragement, moral and spiritual support.

Special thanks also go to the Technicians: Mr. Jefri Samin (FESEM, FKM), Mr Faizal (FESEM, Ibnu Sina), Mr. Subri (ICP-MS, CEPP), Ms Nadirah (Biotechnology Lab), Akira (XPS, Meiji University), Hide & Murakoshi (XRD, Meiji University) and Baba (Contact Angle, Meiji University) for their immense assistance during the laboratory analyses.

I am also highly indebted to my sisters who took care of my kid whenever I was away. I am also thankful to my parents, Hj Basri Hj Ridwan and my mother Hjh Saniah Semat for their prayers and support. I am most thankful for the prayer support and good wishes of my family members and friends.

My kid, Muhammad Daniel Md Kharol who suffered a lot in my absence; I dedicate this thesis to you.

Finally, I would like to express my most profound gratitude to my husband, Md Kharol A. Rahman for encouraging me to continue my education, his prayers and good wishes and for the numerous phone calls and text messages to assure me of his love and support.
May Allah remember the people I might have forgotten and reward them for all the help they gave me.

ABSTRACT

The objective of this study was to develop and characterize the polyethersulfone (PES) incorporated with silver (Ag) as an antibacterial membrane which can remove and disinfect bacteria in a single step for environmental application. The PES-Ag membrane was developed from PES, silver nitrate as an antibacterial agent and 2,4,6-triaminopyrimidine (TAP) as compatibilizer. The influence of $AgNO_3$ loading, molecular weights (MW) of polyvinylpyrrolidone (PVP) as dispersant and type of compatibilizer have been investigated. The resulting membranes were characterized based on their thermal, tensile and structural properties which were used in correlation with the membrane antibacterial properties. The incorporation of Ag in PES membrane has increased the tensile strength doubled as compared to the unmodified PES. Furthermore, it was observed that the highest $AgNO_3$ loading (2 wt%) and the highest MW (360,000) of PVP as dispersant has led to higher silver content on membrane surfaces. This is evidenced from energy dispersive X-ray (EDX) analysis and X-ray photoelectron spectroscopy (XPS). These properties have induced a better antibacterial activity in a disc-diffusion test against *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*). The structural characterization by field emission scanning electron microscope (FESEM) revealed that by incorporating TAP as compatibilizer, smaller Ag particles size with improved distribution and average pore size of 0.174 µm was obtained. In addition, the silver residue during fabrication monitored by inductive coupled plasma-mass spectrometry (ICP-MS) was significantly reduced (62.6%). These parameters have led to *E.coli* removal of log reduction value (LRV) 3.59 and 100% growth inhibition tested on *E.coli* suspension of 1×10^6 colony forming unit (CFU/mL). From the adhesion test, this membrane exhibited the least *E.coli* adherence which in turn evidenced its anti-adhesion property. In conclusion, the PES-Ag membrane with TAP as compatibilizer produced was potential in bacteria removal and disinfection below the CFU maximum range for water and waste water treatment.

ABSTRAK

Objektif kajian ini ialah untuk membangun dan mencirikan selaput (membran) poliethersulfona (PES) yang digabungkan dengan perak (argentum) sebagai selaput anti-bakteria yang akan dapat menyingkir dan menyahjangkit bakteria dalam satu langkah untuk aplikasi alam sekitar. Membran PES-Ag dibangunkan daripada PES, garam nitrat perak ($AgNO_3$) sebagai agen anti-bakteria dan 2,4,6-triaminopyrimidine (TAP) sebagai bahan bantu serasi. Pengaruh muatan $AgNO_3$, berat molekul polivinilpirolidone (PVP) yang bertindak sebagai bahan bantu serak (dispersant) dan jenis bahan bantu serasi (compatibilizer) juga telah dikaji. Membran yang terhasil dicirikan bagi menilai sifat terma, kekuatan tegangan (tensil) dan struktur yang kemudiannya dikorelasi kepada sifat anti-bakteria membran tersebut. Penggabungan Ag ke dalam membran PES telah meningkatkan kekuatan tegangan membran dua kali ganda berbanding membran PES tidak terubahsuai. Muatan $AgNO_3$ tertinggi (2 wt%) dan PVP pada berat molekul tertinggi (360,000 Da) didapati telah berjaya menghasilkan kandungan Ag yang lebih tinggi. Ciri ini telah dibuktikan melalui analisis yang menggunakan kaedah spektroskopi penyebar tenaga sinar-X (EDX) dan kaedah spektroskopi elektron-foto sinar-X (XPS). Sifat-sifat ini seterusnya telah mencetuskan sifat anti-bakteria yang lebih baik, dibuktikan melalui ujian pembauran-cakera (disc-diffusion) terhadap bakteria *Escherichia coli* (*E.coli*) dan *Staphylococcus aureus* (*S.aureus*). Pencirian struktur dengan menggunakan mikroskopi imbasan elektron pemancaran medan (FESEM) telah memberi maklumat bahawa dengan menggunakan TAP sebagai bahan bantu serasi, partikel Ag yang lebih kecil dengan taburan yang lebih baik pada saiz liang purata 0.174 μm telah dperolehi. Di samping itu, sisa Ag yang terlarut resap (leach) semasa pembuatan membran yang dikawal dengan menggunakan spektrometri jisim-berganding plasma teraruh (ICP-MS) didapati menurun dengan nyata sebanyak 62.6%. Keseluruhan parameter yang dikaji telah menunjukkan bahawa penyingkiran *E.coli* adalah pada nilai penurunan log (LRV) 3.59 dan 100% perencatan pertumbuhan apabila diuji pada 1×10^6 unit koloni terbentuk per mL (CFU/mL). Membran ini juga didapati menunjukkan lekatan bakteria (bacterial adherence) yang terkecil dalam ujian lekatan terhadap *E.coli* sekaligus membuktikan sifat anti-lekatan. Kesimpulan daripada kajian ialah membran PES-Ag dengan TAP sebagai bahan bantu serasi adalah sangat berpotensi dalam penyingkiran dan perencatan bakteria di bawah julat CFU untuk air dan rawatan air.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MF	-	Microfiltration
DBPs	-	Disinfection by-products
DOM	-	Dissolved organic matter
UF	-	Ultrafiltration
LRV	-	Log-reduction value
UV	-	Ultra-violet
E.coli	-	*Escherichia coli*
S.aureus	-	*Staphylococcus aureus*
DNA	-	Deoxyribonucleic acid
TGA	-	Thermogravimetric analysis
DSC	-	Differential scanning calorimetry
XRD	-	X-ray diffraction
XPS	-	X-ray photoelectron spectroscopy
EDX	-	Energy dispersive X-ray
ICP-MS	-	Inductive coupled plasma-mass spectrometer
CFU	-	Colony forming unit
NOM	-	Natural organic matter
DOC	-	Dissolved organic carbon
SS	-	Suspended solids
TDS	-	Total dissolved solid
pH	-	$-\log[H^+]$
WHO	-	World of Health Organization
DLVO	-	Deryaguin-Landau and Vervey-Overbeck
MWCO	-	Molecular weight cut-off
Da	-	Dalton
AFM	-	Atomic force microscopy
ATR-FTIR	-	Attenuated total reflection-Fourier transform infra-red spectroscopy
FESEM	-	Field emission scanning electron microscope
ppt	-	Part per trillion
ppm	-	Part per million
PWP	-	Pure water permeation
TOC	-	Total organic carbon
MW	-	Molecular weight
NA	-	Nutrient agar
OD	-	Optical density
MD	-	Minimal Davis
rpm	-	Revolution per minute
v/v	-	Volume per volume
Fig.	-	Figure
MTS	-	Mechanical testing system

Wt.	-	Weight
Eq.	-	Equation
SD	-	Standard deviation
DMFC	-	Direct methanol fuel cell
cps	-	Count per second
BE	-	Binding energy

LIST OF SYMBOLS

%	-	Percent
˚C	-	Degree Celcius
h	-	hour
mA	-	miliampere
kV	-	Kilo Volt
°	-	degree
θ	-	theta
μM	-	Micro molar
wt. %	-	Weight percent
d	-	Diameter
J_v	-	Pure water permeation (Flux)
r_m	-	Mean pore diameter (μm)
ε	-	Porosity (%)
Q	-	Volume of permeate per unit time (m^3s^{-1})
A	-	Membrane surface area (m^2)
ΔT	-	Permeation time (s)
μm	-	Micrometer
nm	-	Nanometer
C_p	-	Concentration of permeate (ppm)
C_f	-	Concentration of feed (ppm)
mg/L	-	Miligrams per litre
mm/min	-	Milimeter per minute
V_R	-	Repulsive energy
V_A	-	Attractive energy
V_T	-	Total energy
T_g	-	Glass transition temperature

LIST OF APENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Microbiological contamination of water sources has long been a concern to the public. According to some authors, there were numbers of various bacterial species available (ranging from 10^2 to 10^4 mL^{-1}) in raw water as well as sewage effluents (Bonnélye *et al*., 2008; Goldman *et al*., 2009) tend to adhere to surfaces and grow mainly at the expense of nutrients accumulated from the water phase. Microbiological contamination in any sources should be avoided at any cost since in the production of potable water, only a limited number of bacteria (depends on the type of bacteria) are acceptable. The process for the removal of contaminants depends not only on the nature of the microorganisms but also on the desired levels of purity.

The use of membrane filtration in water treatment has greatly contributed to greener technology. For example, microfiltration (MF) membrane has been widely applied in water purification process due to its capability to remove microorganisms and to treat harmful pollutants as well as dissolved organic matters (DOM) (Ghayeni *et al*.,1996; Oh *et al*., 2007). Laine *et al.* reported that ultrafiltration (UF) applications represent 74% of the total installed low-pressure membrane full-scale plants (identified world-wide) in water industry in order to meet more stringent regulations in producing drinking water (Laine *et al*., 2000).

It was reported in the open literature that membrane technology is one of the disinfection technique where microorganisms are retained without any chemicals engagement. However, the problem of biofouling aroused when membrane is applied, due to the accumulation of microorganisms on membrane surfaces. In addition, the current practice of membrane filtration required additional step addressed as disinfection step via techniques such as chlorination (the most common one), ozonation and UV. There were many and thorough discussion available in the use of chlorination recently. The use of chlorination may lead to the disinfection by-products (DBPs) release which in turn exposed consumers to potential carcinogenic compounds such as the derivatives of chloramines.

Many studies have been conducted to overcome/meet the restrictions as well as to resolve membrane fouling problems due to the uncontrolled accumulations of micro-species. In handling biofouling problems, membrane modification, low-flux operation and chemical cleaning are areas to be explored (Chang *et al*., 2002; Khor *et al*., 2007). The effective prevention of microbial growth in a membrane system can only be achieved when continuous and sufficiently high chlorine concentration is maintained. However, due to stricter legislative regulation on chlorine usage, other effective and environmental-friendly alternative is needed.

In membrane modification, the research in combining inorganics into polymer matrices has been expanding since 1990-s. The inorganics chosen were tailored with the application such as catalysis, biochemistry, separation and sensing. In gas separation, the inorganic fillers namely zeolite, carbon molecular sieve, silica and metal oxides has contributed to enhance membrane separation performance specifically in addressing flux decline and selectivity (Rafizah *et al*., 2008; Kusworo *et al.*, 2008; Ismail *et al*., 2009; Mataram *et al*., 2010).The combination of inorganics in polymer matrices or well-known as mixed-matrix provides the solution for highly cost-maintenance and brittleness of inorganic membranes. The inorganic fillers in mixed matrix membrane act to create preferential permeation pathways for selective permeability while posing a barrier for undesired permeation in order to improve the separation performance (Goh et al., 2011).

In water application, attempts were made in addressing flux decline due to the accumulation of (micro- or macro-)species onto membrane surfaces which in turn affecting the separation performance. The important issue in membrane manufacturing is to develop membrane with suitable pore size in order to attain various sizes of contaminants. In addition, membrane must also show sufficient resistance towards the feed components as well as the operating condition. In antibacterial application, a number of researches have been conducted in exploring silver-incorporation to polymeric materials such as cellulose acetate (CA), polacrylonitrile (PAN), polysulfone (PSf) and chitosan for the application of water treatment, nano-fibre and food-packaging (Chou *et. al*., 2005; Wang *et. al*., 2005; Ma *et. al*., 2008, Zodrow *et. al*., 2009). Silver was found to leach in the reported articles and to date; attempts on overcoming this problem are still not published in the open literature.

1.2 Problem statements

The conventional UF membrane in water and waste water treatment established in bacteria removal has achieved a promising rejection value of >99% or to be specific, a log reduction value (LRV) of >3. However, after the membrane filtration process, it is necessary to perform an extra step which is disinfection as a secondary bacteria control barrier and distribution system protection (Ghayeni *et al*., 1996; Zio *et al*., 2005). The options available for disinfection are: UV, ozonation and chlorination. The most commonly used method is chlorination due to the easy-handling process and cost effectiveness. However, the major drawbacks of this method is when greater number of bacteria present, higher concentration of chlorine is needed, hence more disinfection by-products (DBPs) will be released in the water distribution system. Current waste water treatment with microbial burden is facing the problem of biofouling due to the accumulation of microorganisms over operation time. In order to address the issues, current research is conducted to explore the possibility and effectiveness of using a UF membrane incorporated with an antibacterial agent in removing bacteria as well as to investigate its potential in behaving anti-biofouling properties.

1.3 Objectives of the study

Based on the existing problem statements, the current study has been performed with the following objectives:

i. To develop an antibacterial membrane by incorporating silver (Ag) as an antibacterial agent without sacrificing membrane fluxes and removal abilities and to characterize the membrane in terms of mechanical, morphological, water permeation, hydrophilicity and pore sizes.
ii. To study the effect of incorporating a compatibilizer, PVP of different molecular weights in membrane properties and performances.
iii. To evaluate the effect of incorporating different compatibilizers in membrane properties and performances.
iv. To evaluate the fluxes of prepared membranes using pure water permeation test on the custom-made test-rig.
v. To evaluate the antibacterial performance of membrane using disc diffusion method, filtration of bacterial suspension and anti-biofouling tests.

1.4 Research scopes

In order to achieve the above mentioned objectives, the following scopes of study were drawn.

i. Preparation of dope using PES as polymeric material, NMP as solvent and $AgNO_3$ as an additive or precursor of antibacterial agent, silver (Ag).
ii. Selection of $AgNO_3$-loading and compatibilizer based on the evaluation in the miscibility and antibacterial tests.
iii. Fabrication of PES-$AgNO_3$ asymmetric membrane using phase inversion technique and characterization of membranes in terms of mechanical strength, hydrophilicity, overall porosity, pore sizes and water permeation.
iv. Evaluation of Ag-entrapment in prepared membranes by using ICP-MS, EDX and XPS techniques.
v. Membrane fluxes measurement was carried out by using custom-made test rig at pressure range 1-6 bar.

vi. Performance measurement of prepared membranes was conducted in terms of antibacterial activity by using disc diffusion method and bacteria removal via the filtration of bacterial suspension.

vii. Performance measurement of prepared membranes was conducted in terms of anti-biofouling properties through an anti-adhesion test.

viii. Comparison of PES pristine membrane with PES antibacterial membrane in all characterization and antibacterial tests.

1.5 Research significance

This study is of significance to the research of water treatment which involves disinfection steps. The antibacterial membrane extends the multi-steps options for water treatment to a stand-alone removal and disinfection of bacteria. The results obtained in the study also provide the information in bacteria-removal and bacteria-killing mechanisms which lead to the most effective options in treating polluted water. Furthermore, the information on silver entrapment obtained in this study would be beneficial to the other related fields such as in medicinal and electrical field where silver is optimized in wound dressings and conducting material.

1.6 Organization of the thesis

The thesis is divided into six chapters. The first chapter presents the research background as well as the problem statement. The research objectives, scopes and significance are also highlighted in first chapter. Chapter two provides the literature review on bacteria removal which includes the theories of the whole process and the options available for bacteria removal. The advantages of antibacterial membrane, current status and future direction of the technology are also discussed in this chapter. Chapter three is dedicated to the detailed description of the research methodology. The material selection for dope preparation, membrane fabrication and performance testing conducted in this work are explained in this chapter. In chapter

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